Folic Acid Supplementation and Neurological Function in a *Drosophila* Model

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Abstract

Alzheimer's is a devastating disease with few treatments.[1] Some studies support the therapeutic effect of folic acid supplementation for Alzheimer's patients.[2] Therefore, two fruit fly strains (W^{1118} and $Appl^{d}$) known for memory defects and two control strains were fed a regular, control diet or a folic acid supplementation diet. Two reflexes, phototaxis and negative geotaxis, were tested as a proxy for nervous system function. Assays were conducted every five days over a twenty day period. Statistical analysis revealed that folic acid supplementation improved climbing performance in one strain.

Introduction

Alzheimer's disease affects 5.6 million Americans and is likely to become more prevalent as our population ages.[1] Current treatments only offer temporary relief of the symptoms.[1] Folic acid has been proposed to alleviate these symptoms through several potential mechanisms, one of which involves upregulating enzymes that methylate DNA, thereby reducing amyloid protein expression.[2] *Drosophila* is a useful model to investigate this role because of its short lifespan, amenability to genetic manipulation, and complex nervous system resembling the one found in vertebrates.[3]

Methods

Fly Stocks: Four strains were used in this experiment: $Appl^{d}$ (an Alzheimer's model strain with a deletion of the fly amyloid protein), stock #38405 (males in this strain lack amyloid and have the genotype Df(1)yT7-518/Dp(1;Y)y[+]m[64]), W^{1118} (a memory-defective white-eyed strain), and wild-type (Table 1).[4] The first three strains were obtained from Bloomington and the wild-type flies came from Carolina. All strains were cultured at 20°C and were fed either standard blue fly media or media supplemented with a 125 µM dose of folic acid for the duration of the experiment. Climbing and phototaxis assays were conducted and videoed on days 0, 5, 10, 15, and 20.

Phototaxis Assay: To test reflexive movement towards light, two vials were connected as shown in Figure 1. Each group was transferred to the apparatus and given five minutes to accommodate in a dark room. After the flies accommodated they were tapped to the bottom of one vial and a light was introduced at the opposing end.[5] After 10 seconds, the flies were recorded as being in either the original vial or the light vial.

Climbing Assay: To test negative geotaxis, or reflexive movement against gravity, each group of flies was transferred to an empty vial, immediately tapped to the bottom, and allowed to climb for 5 seconds (Figure 2).[6] Each test day, seven tests separated by one-minute intervals were done for each treatment group within each strain. The position of each fly was recorded to the nearest millimeter. Flies were grouped into bottom (0-2.5 cm), middle (2.6-5.5 cm), and top (5.6-8.5 cm).

Statistical Analysis: Proportions were transformed using the arcsine method. In R Studio, normality was tested using QQ plots and homogeneity of variances via Brown-Forsyth tests. Two-way ANOVAs were used to examine the impact of strain on assay performance over time and of treatment on assay performance within each strain.

$Appl^{d}$	38405	W^{1118}	Wild Type
Memory defects	$Appl^{d}$ Progenitor	Memory defects	W^{1118} Control

Table 1: Strain Selection

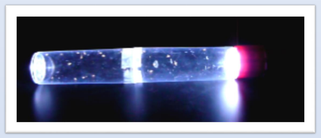

Figure 1: Phototaxis assay

Figure 2: Climbing assay

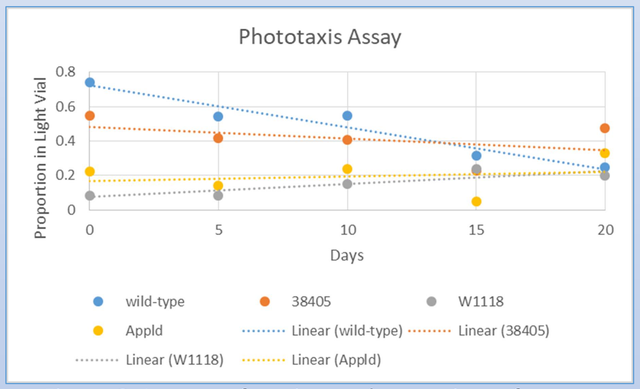

Figure 3: Impact of strain on phototaxis performance

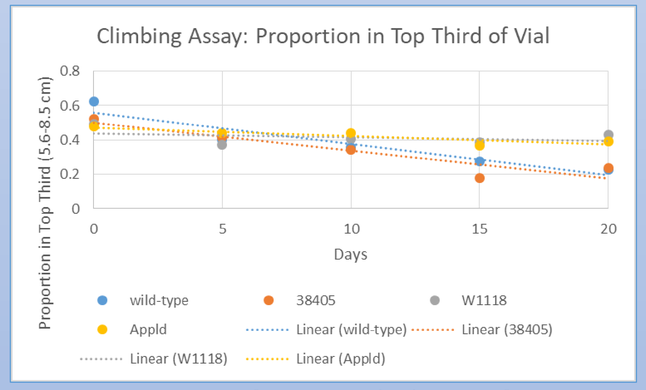

Figure 4: Impact of strain on climbing performance

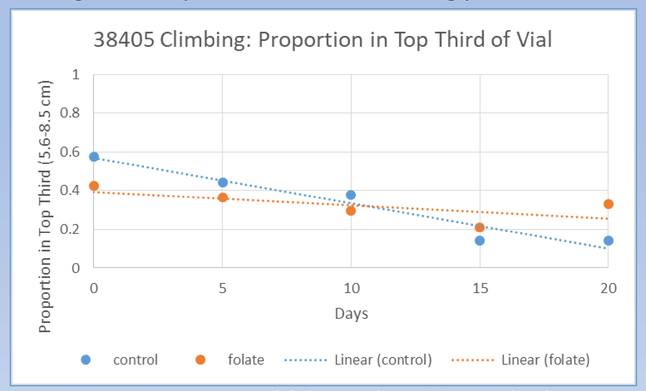

Figure 5: Impact of folic acid on 38405 Climbing

Results

A comparison of performance of all strains on the phototaxis assay revealed that wild-type performance declined over time (p=0.01), while W^{1118} performance improved (p=0.004) as shown in Figure 3. Out of the three segments of the climbing assay, the only statistical difference in performance over time between the strains was observed in the top portion. Only W^{1118} did not experience decline over time (Figure 4). No significant difference was observed between control and folate groups over time within any of the four strains in the phototaxis assay. For the climbing assay, out of all the strains and the three segments, the only significant impact of treatment was observed in the proportion of 38405 flies in the top. The control group significantly declined over time (p= 0.006), but the folate group did not significantly change over this same time period (p=0.2) as demonstrated in Figure 5.

Conclusion

W^{1118} flies appear to exhibit less neurological decline than the other three strains included in this study. Given that this strain is reported to have memory defects, this finding suggests that this strain either requires more time to degenerate or that the assays used may not accurately reflect memory. Folate may have a therapeutic effect in slowing the neurological decline of 38405 flies over time. It is possible that detecting a significant difference for other strains would require testing more flies over a longer time period.

Acknowledgements

This project was funded by an A.S.U. Undergraduate Faculty-mentored Research Grant.

Dr. Nicholas Negovetich assisted with statistical analysis.

Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study.

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